## I. AMENDMENTS TO THE SPECIFICATION

Please replace paragraphs as filed with the following replacement paragraphs:

**[0016]** FIG. 6A depicts diagrams of the structures of APP, the neurexin 1β/APP hybrid protein (Nrx1β/APP), and the modified neurexin 1β (Nrx1β*) containing an insertion including the 7 amino acid β –secretase binding site sequence: EVKMDAE (SEQ ID. NO:1) from APP into neurexin lβ just N-terminal to the TMR.

**[0029]** One embodiment of the present invention provides compositions which are useful in the screening and identifying of an agent or agents that modulate the cleavage of APP. One composition of the present invention comprises a polypeptide substrate for cleavage by β-secretase, wherein the polypeptide substrate includes a transmembrane region and an exogenous APP β –secretase cleavage site inserted near the transmembrane region. In certain embodiments of the present invention, the exogenous β-secretase cleavage site comprises the amino acid sequence EVKMDAE (SEQ. ID NO:1) Alternative embodiments of the present invention also include nucleic acid sequences encoding the polypeptide substrate.

**[0030]** Another embodiment of the compositions provided by the present invention comprises a polypeptide substrate including a transmembrane region and an exogenous APLP1 or APLP2 β-secretase cleavage site inserted into the polypeptide near the transmembrane region. In certain embodiments, the insertion includes the exogenous APLP1 β-secretase cleavage site that is included in the APLP1 linker region sequence DELAPAGTGVSRE (SEQ. ID NO: 2) and the analogous APLP2 β-secretase cleavage site. Alternative embodiments of the present invention also include nucleic acid sequences encoding the polypeptide substrate.

**[0031]** One embodiment of the present invention provides a method of screening and identifying agents that modulate the cleavage of APP by β-secretase comprising: providing a chimeric molecule, wherein the chimeric molecule includes a transmembrane domain, a β-secretase cleavage site, and an extracellular domain; contacting the chimeric molecule with a β-secretase in the presence or absence of potential cleavage modulating agents; and identifying occurrences of cleavage of the chimeric molecule, wherein a difference in cleavage in the presence of the agent relative to cleavage in the absence of agent is indicative of a cleavage modulating agent. In some embodiments of this method, the β-secretase cleavage site comprises the amino acid sequence EVKMDAE (SEQ. ID NO:1), while in alternate embodiments the β-secretase cleavage site would include an amino acid sequence which corresponds to the amino acid sequences of APLP1 or APLP2 that are responsible for mediating β-secretaase cleavage. In certain embodiments, the β-secretase cleavage site will comprise the amino acid sequence DELAPAGTGVSRE (SEQ. ID NO:2).

**[0041]** Another embodiment of the present invention provides methods of identifying an agent or agents that differentially modulate the cleavage of APP in comparison to the cleavage of APLPs. One such embodiment provides a method of screening and identifying agents that modulate the cleavage of APP by β-secretase comprising: providing an APP chimeric molecule, wherein the APP chimeric molecule includes a transmembrane domain, an APP β-secretase cleavage site, and an extracellular domain; providing an APLP chimeric molecule, wherein the APLP chimeric molecule includes a transmembrane domain, an APLP β-secretase cleavage site and an extracellular domain; contacting both chimeric molecules with a β-secretase in the presence or absence of potential modulating agents; and differentially identifying occurrences of

cleavage of the chimeric molecules. In these embodiments, the APP β-secretase cleavage site comprises the amino acid sequence EVKMDAE (SEQ. ID NO:1), while the APLP β-secretase cleavage site would include an amino acid sequence which corresponds to the amino acid sequences of APLP1 or APLP2 that are responsible for mediating β-secretase cleavage. In certain embodiments, the β-secretase cleavage site will comprise the amino acid sequence DELAPAGTGVSRE (SEQ. ID NO:2).

**[0054]** Most plasmids used for the transactivation assays, such as pG5E1B-luc (Gal4 reporter plasmid), pCMV-LacZ (β-galactosidase control plasmid), pM-Tip60 (expression of Gal4-Tip60 fusion protein), pCMV-Fe65 and pCMV-APP (APP), were reported previously (Cao, X., and Südhof, T.C. (2001 Science 293, 115-120). pcDNA3.1-APLP2 encoding APLP2 was constructed by inserting a 3.0 kb DNA fragment from pOTB7-hAPLP2 (Image clone ID:2820109) into the BamHI and XbaI sites of pcDNA3.1.pCMV-Sport6-APLP1 encoding APLP1 was acquired from ATCC (Image ID:3865417). pCMV-SynCam and pCMV-Nrx1 β were described previously (Biederer, T., et al. (2002) Science 297, 1525-1531, Ushkaiyov, Y.A., et al. (1994) J. Biol. Chem. 269-11987-11992). pCMV-Nrx1β-BS encoding neurexin 1β with 7 residues from APP was generated by inserting synthetic oligonucleotides (sequences: QL0311 = GGC CGA GAA GTG AAG ATG GAT GCA GAA AGC (SEQ. ID NO:3); QL0312 = GGC CGC TTT CTG CAT CCA GGT TCA CTT CTC) (SEQ. ID NO:4) into the Not 1 site of pCMV-Nrx1β. pCMV-NrxAPP was constructed by ligating a 0.6kb fragment encoding residues 494-695 of $APP_{695}$ into the Not 1 site of pCMV-Nrx1β. pcDNA3.1-ADAM 9 encodes ADAM 9 and pcDNA3.1-Myc-BACE encodes BACE.

**[0055]** APP, APLP1, and APLP2 antibodies were raised in rabbits to synthetic peptides containing their C-terminal sequences (APPc = U955: GYENPTYKFFEQMQN (SEQ. ID NO:5); APLP1c = U2976: YENPTYRFLEERP SEQ. ID NO:6; APLP2c = U2977: NKMQNHGYENPTYKYLEQMQI (SEQ. ID NO:7))and to an interior peptide from APLP1 (APLPIx = U4787; residues 554-567: VPRGEPFHSSEIQR (SEQ. ID NO:8)). Monoclonal antibodies to the extracellular domain of APP (5A3 and 1G7[38]) were a kind gift of E.H. Koo (La Jolla, CA). All other antibodies were described previously (Cao, X. and Südhof, T.C. (2001) Science 293, 115-120; Biederer, T., et al. (2002) Science 297, 1525-1531;Ushkaiyov, Y.A., et al. (1992) Science 257, 50-56).

**[0064]** The cleavage of APLP1 and APLP2 by BACE was unexpected considering the lack of sequence homology in the APP cleavage site for BACE 1 (Fig. 1B). Although the co-transfection/cleavage assay for BACE 1 cleavage has been used previously to confirm BACE 1 enzyme activity, the specificity of BACE 1-mediated cleavage under these conditions has not been investigated, and the substrate specificity of BACE 1 has been studied primarily with short synthetic peptides. The data in Fig. 4 demonstrate that BACE 1 does not randomly cleave overexpressed cell-surface proteins since neurexin 1β and SynCAM were not digested. However, the data raises the question of what determines the cleavage specificity of BACE 1: is cleavage a property of a specific sequence in APP, possibly in combination with the proximity of that sequence to the membrane, or are the extra- or intracellular domains of APP involved in directed BACE 1 cleavage? To address these questions, a hybrid neurexin 1β/APP protein in which the extracellular sequences of neurexin 1β are fused to the C-terminal sequences of APP at a position just N-terminal to the normal BACE 1 cleavage site was constructed. Figure 6A, shows

diagrams of the structures of APP, the neurexin 1β/APP hybrid protein (Nrx1β/APP), and the modified neurexin 1β (Nrx1β*) containing a seven residue insertion (sequence: EVKMDAE (SEQ. ID NO:1)) from APP into neurexin 1β just N-terminal to the TMR. The β-secretase cleavage site containing the Swedish mutation EVNLDAES (SEQ. ID NO:9) that is preferentially cleaved by BACE 1 was also inserted. In the neurexin 1β/APP hybrid protein, the nearly complete extracellular sequence of neurexin 1β (residues 1-375) is fused to APP such that only a short sequence of the extracellular linker from APP (... residues) followed by the TMR and AICD from APP are included (residues 494-695 of $APP_{695}$). Comparison of the cleavage of APP and of the neurexin/APP hybrid protein in transfected cells revealed that they were almost identically processed, with a similar production of CTFs that were significantly shifted to larger sizes by co-expression of BACE 1. Figure 6B shows a comparison of the effect of BACE 1 and DAPT on CTFs produced in HEK293 control cells (lanes 1-2) or transfected cells expressing APP (lanes 3-6) or the neurexin 1β/APP hybrid (lanes 7-10). Cells transfected and treated as indicated were examined by immunoblotting after standard gel electrophesis (top panel) or tricine gel electrophoresis (bottom panel) with antibodies to the C-terminus of APP. For both APP and the APP/neurexin hybrid, DAPT induced a massive accumulation of CTFs consistent with normal digestion of the CTFs by γ-secretase (lanes 4, 6, 8, and 10). Thus the extracellular domains of APP are dispensible for BACE 1 cleavage.

**[0065]** The ability of intracellular AICD or the TMR of APP to direct BACE 1-was tested by inserting a seven residue sequence from APP that encompasses the normal BACE 1 cleavage site sequence: EVKMDAE (SEQ. ID NO:1) into full-length neurexin 1β. The insertion was placed just outside of the TMR, corresponding to the normal position of this sequence in APP, but no

other APP-related sequences were present in this neurexin 1β derivative (referred to as Nrx1β*). Analysis of co-transfected cells revealed that insertion of the short 7 residue sequence was sufficient to convert neurexin 1β into a BACE 1 substrate (Fig. 6C; same as B., except that the effect of BACE 1 on the cleavage of neurexin 1β and neurexin 1β* was examined): Now CTFs are observed as a function of BACE 1 in the absence of DAPT (compare lanes 7 and 9), and the amount of CTFs is greatly increased by co-expression of BACE 1 in the presence of DAPT (lanes 8 and 10). BACE 1 cleavage of Nrx1β* was so efficient that the amount of full-length Nrx1β* protein dropped precipitously in cells co-transfected with BACE 1 (Fig. 6C). These data demonstrate that even in the context of a resident membrane protein, a very short substrate sequence is sufficient to confer BACE 1 cleavage onto a protein.

## II. ADDITION OF SEQUENCE LISTING

Please place the attached paper copy of the "Sequence Listing" in the captioned application beginning as a new page after the "Abstract of the Disclosure."